Stopping death cold

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The three-dimensional structure of Bcl-x_L, an inhibitor of apoptosis, suggests how different combinations of proteins in the same family might be utilized to control apoptosis.

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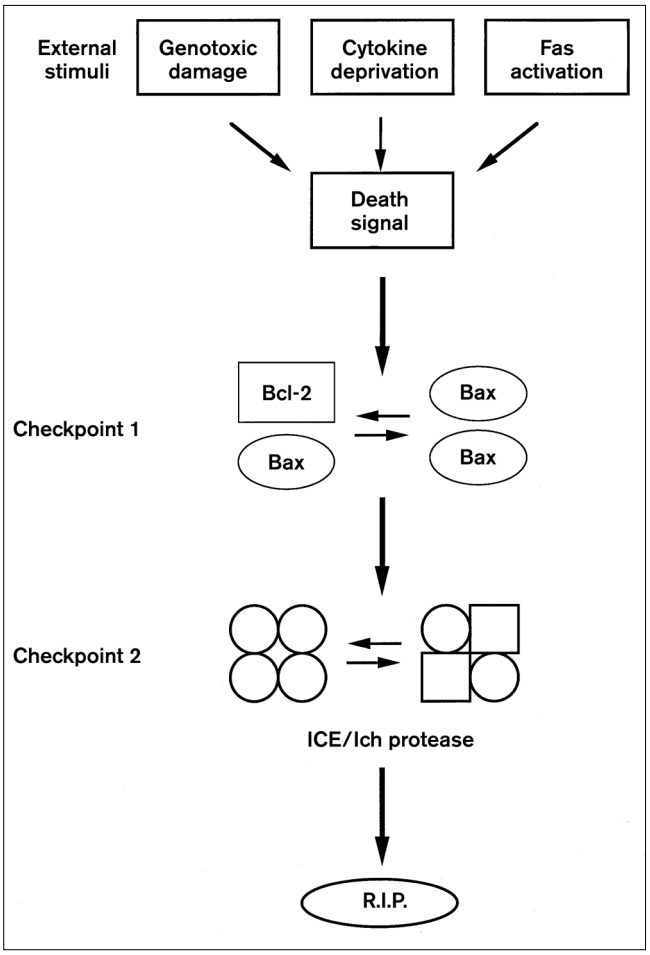
Programmed cell death (PCD) or apoptosis plays a fundamental role in the development and maintenance of cellular homeostasis. Homologous proteins and pathways in apoptosis are found in many species, from worms to humans; thus, regulating cellular demise appears to be a critical activity in the life (and death) cycle of the cell in all organisms. Although extracellular stimuli play a principal role in switching on the cell-death response, the sensitivity of a given cell to such stimuli is clearly cell-type specific [1]. Checkpoints seem to exist along the pathway to cellular suicide, which are mediated by two different protein families (Fig. 1) [2]. The primary checkpoint appears to comprise the Bcl family of proteins, members of which can act as repressors (A1, Bcl-2, Bcl-x_L, Mcl-1) and effectors (Bax, Bad, Bcl-x_S) of apoptosis. The use of alternate combinations of Bcl family members appears to have the capability to either promote or block transmission of a cell-death signal, although the intervening transducers of this information have yet to be identified. The three-dimensional (3D) structure of a death repressor from the Bcl family, Bcl-x_L, illustrates how different patterns of oligomerization might act as a point of decision, a sort of cellular 'to be or not to be' [3]. The secondary checkpoint affects the foot soldiers of apoptosis itself namely, the activity of the cysteine proteases (interleukin-converting enzyme [ICE] and Ich [the analogous enzyme from worms] proteases). These proteases presumably degrade select proteins, ultimately destroying the means of their own survival (Fig. 1). The extent of generalized proteolysis is not presently known.

Figure 1

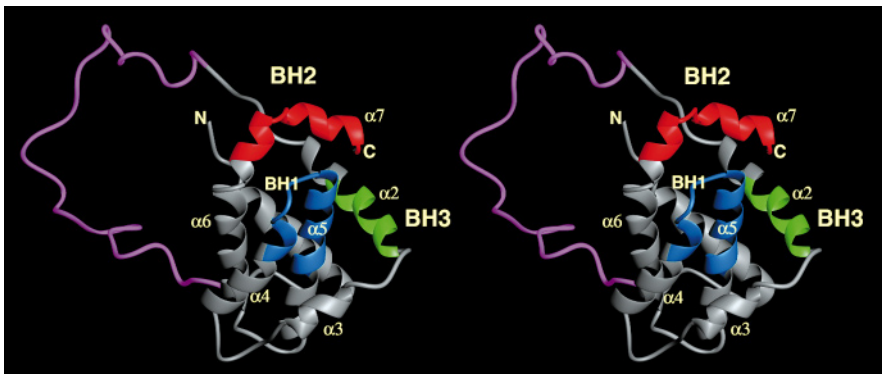


Schematic pathway of cellular suicide. External stimuli initiate a program of cellular suicide that is regulated at two checkpoints. The initial intracellular death signal has yet to be identified and the number of steps leading to Checkpoint 1 remains to be determined. The first checkpoint represents the exchange of apoptotic repressors (e.g. Bcl-2) with apoptotic effectors (e.g. Bax). Conversion of a Bax/Bcl-2 heterodimer to a Bax homodimer, for example, results in stimulation of apoptosis and continuation to the next checkpoint in the PCD pathway. Checkpoint 2 represents the interchange of subunits of ICE or Ich proteases, which may result in a functional cysteine protease. Only a properly constructed heterotetramer leads to an active enzyme. It is presently unknown if there are any intervening regulatory checkpoints between the Bcl and ICE/Ich protease checkpoints illustrated. (The figure was adapted from [2], with permission.)

The Bcl family

Bcl-2, the founding member of the Bcl family, was originally identified at the t(14;18) breakpoint in follicular B-cell lymphoma [4]. The family is characterized by three short regions of sequence similarity termed Bcl homology (BH) domains 1, 2 and 3 (Fig. 2) [5]; each domain comprises only 15 to 20 amino acids of the »200 amino acids in the full-length protein. The Bcl family comprises both effectors and repressors of apoptosis; in addition, altered splicing events are responsible for the dual role observed for at least one member known as Bcl-x. The full length

Figure 2



Stereoview showing the arrangement of the seven helices in the three-dimensional structure of Bcl-x_L. The three conserved domains are BH1 (blue), BH2 (red) and BH3 (green). The large disordered loop is shown in purple.

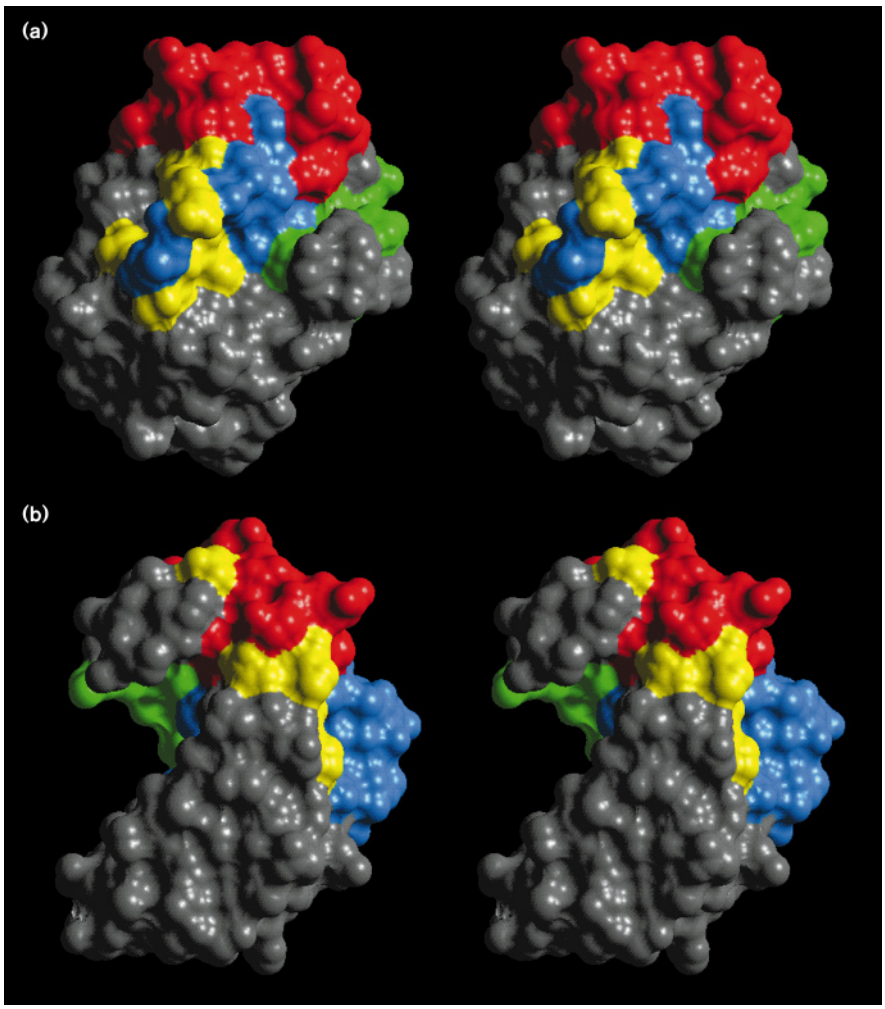
molecule, Bcl-x_L (for long), represses apoptosis whereas a truncated form of the protein lacking most of the BH2 domain, known as Bcl-x_S (for short), acts as an apoptotic effector [6].

The 3D structure of Bcl-x_L [3], (in which the putative transmembrane region at the C terminus is absent) reveals a predominantly helical protein in which two centrally located, primarily hydrophobic α helices (α5 and α6) are surrounded by five amphipathic helices (Fig. 2). BH1 and BH2 are constructed as helix-loop-helix modules in which the loop forms an irregular structure. In conjunction with the third conserved domain, BH3, the three homology regions form an elongated hydrophobic cleft which may form a surface for interaction with other Bcl family members. Between α1 and α2 there is a highly disordered loop of 52 amino acids (Ser28 to Val80). This segment fails to display electron density in single crystals and no medium or long-range NOEs are seen in NMR spectra in solution that could be used to define its structure. For deletion mutations in which some or all of this loop is replaced by a tetrad of alanines, the anti-apoptotic activity of Bcl-x_L is not substantially altered upon interleukin-3 (IL-3) withdrawal (one means of inducing apoptosis in cell culture). The biological role of this loop therefore remains a mystery. It may be that it is induced to form a structural interface when presented with an as yet unidentified component of the PCD machinery, perhaps in response to some death signals but not to others.

Genetic and molecular analyses of the BH domains indicated that these conserved regions play an important role in defining the biological consequence of *bcl* gene expression. Selected mutations in either the BH1 or BH2 domains of Bcl-2 disrupt the ability of Bcl-2 to heterodimerize with the apoptotic effector Bax, thereby destroying the ability of Bcl-2 to block apoptosis [7,8] (Fig. 3). But these mutants do not block the ability of Bcl-2 to homodimerize, although no biological significance has been attributed to homodimerization, to date. Homodimerization occurs, possibly in a head-to-tail orientation, via sequences in the N- and C-termini [8,9]. It is interesting to note that some mutations in Bcl-2 that disrupt heterodimerization with Bax and abrogate the ability of Bcl-2 to inhibit apoptosis do not have the same effect in Bcl-x_L. For example, replacement of the segment Phe138–Arg–Asp–Gly141 in BH1 of Bcl-2 (131–134 of Bcl-x_L) disrupts the interaction of Bcl-2 with Bax with concomitant loss of cell viability upon IL-3 withdrawal induced apoptosis [7]. But point mutants across this identical segment in Bcl-x_L, which block heterodimerization with Bax, do not substantially diminish cell viability after apoptotic induction with Sindbis virus [10]. Thus, the functional significance of the BH domains seems to vary among family members. The 3D structure of Bcl-x_L provides the opportunity to conduct more detailed functional mapping of the protein to define the true limits of these domains and to identify whether they indeed form structural units within the protein. Once delimited, similar efforts with different family members may lead to better definitions of the characteristics of individual BH domains that distinguish one protein from another.

Competing equilibria for different dimerizations among Bcl family members has been proposed to form the basis of a molecular rheostat for apoptotic regulation [2] (Fig. 4). Formation of different partners can therefore be used to stimulate or suppress cellular suicide, as in the first checkpoint on the PCD pathway. Careful balancing of the ratios of effector or repressor combinations has already been shown to form the basis of this switch for a p53-initiated death program [11]. There are hints that a similar process occurs at the secondary checkpoint, in assembling the active, heterotetrameric form of ICE proteases [12,13]. We see evidence, therefore, that a scheme involving alternative patterns of protein oligomerization has the potential to be a powerful regulatory mechanism. Indeed, this mechanism was recognized long ago to be important in controlling gene expression. Two different transcription-factor families contain a similar dimerization interface known as a leucine zipper. The extent of transcriptional activation is

Figure 3



The putative binding pocket of Bcl family members. Molecular surface representations of Bcl-x_L are shown with the conserved domains colored as in Figure 2. BH1 residues in Bcl-2 whose mutation disrupts interaction between Bcl-2 and Bax have been mapped onto the molecular surface of Bcl-x_L. **(a)** These include residues Phe138–Gly141 and Trp144–Arg146 of Bcl-2 (Phe131–Gly134 and Trp137–Arg139 of Bcl-x_L), which have been highlighted in yellow. **(b)** View shown rotated approximately 90° about the vertical axis relative to that in (a). The residues shown here are Trp188, Gln190–Asp191 and Glu200 of Bcl-2 (Trp181, Gln183–Asp184 and Glu193 of Bcl-x_L), highlighted in yellow. It is not known if it is only mutations in these amino acids that can disrupt interaction between Bcl-2 and Bax.

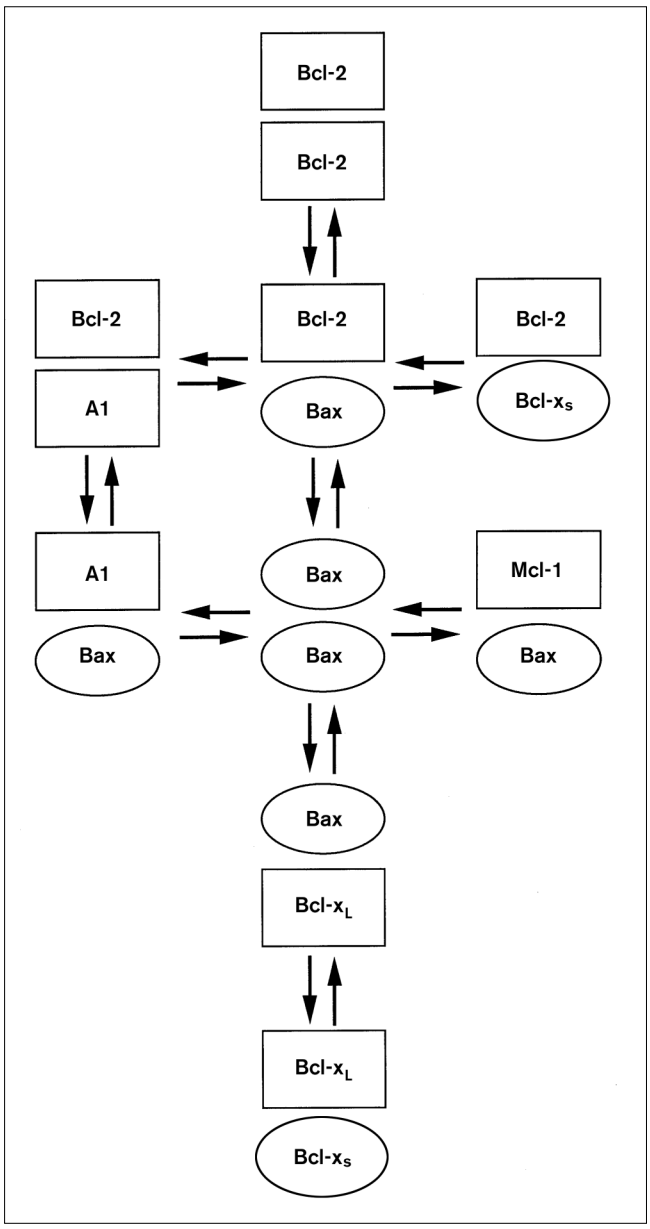
regulated, at least in part, by alternative pairing of protein monomers that are capable of sequence-specific recognition of DNA as either homodimers or heterodimers [14]. The delivery of different DNA recognition surfaces is mediated by the specificity of pairing determined at the dimerization interface formed by the leucine zipper. In a like manner, the BH1 and BH2 domains in the Bcl family can permit formation of different combinations of proteins that are capable of stimulating or repressing cellular suicide (Fig. 4). What is needed is the identification of a signalling surface that transmits the death signal beyond the Bcl checkpoint. Is this simply a nonconserved domain in a Bcl protein or is there another class of signalling molecule with which a Bcl oligomer must interact to stimulate the assembly or activity of ICE or Ich proteases?

Points of influence

Although absent in the structural effort of Fesik and coworkers [3], Bcl-x_L and Bcl-2 possess a hydrophobic tail capable of membrane insertion. Bcl-2 can be anchored to mitochondrial and microsomal membranes via the hydrophobic tail, with the balance of the protein facing the cytosol [15,16]. Interestingly, there is a structural relationship between Bcl-x_L and the pore-forming domain of diphtheria toxin (Fig. 5). This domain of diphtheria toxin is thought to dimerize and insert into the cell membrane, thereby dissipating electrochemical gradients across a biological membrane [17]. Bcl-2 has recently been shown to localize to the perinuclear membrane and to the endoplasmic reticulum (ER), in addition to its localization to mitochondria. Moreover, Bcl-2 directly or indirectly regulates Ca^{2+} flux across the ER membrane [18]. The ability of Bcl-2 to associate with different membranes and its structural relationship to diphtheria toxin (by analogy with the structure of Bcl-x_L) raises the intriguing possibility that Bcl-2 may receive or even intercept cell-death signals mediated by ion gradients [3].

Despite all of these attractive possibilities, the *in vivo* roles of Bcl proteins have remained elusive. Bcl-2 has a

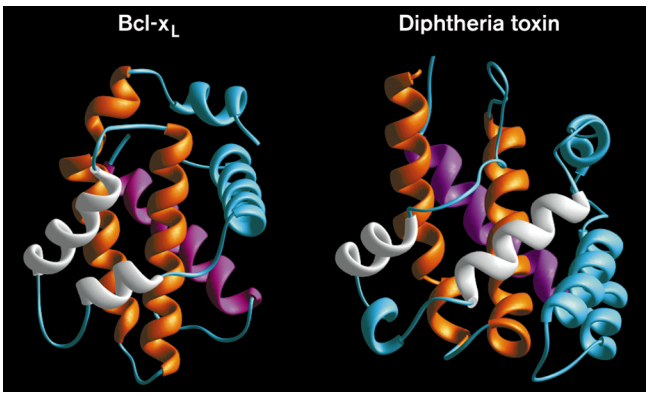
Figure 4



Hypothetical scheme for the exchange of death effectors and repressors of the Bcl family at Checkpoint 1 in the PCD pathway. Effectors are indicated with ellipses, repressors with squares. The interchange of subunits depicted is derived from the observation of pairwise interactions for these proteins observed in a yeast two-hybrid assay. The Bcl-2 and Bax homodimers and the Bcl-2/Bax, Bax/Bcl-x_L heterodimers have also been proven to occur in mammalian cells. No significance is attached to the positioning of Bcl family members in the diagram. Current understanding of the function of the different dimeric species suggests that all heterodimeric combinations with Bax lead to apoptotic repression. All other combinations do not presently have a defined phenotype. (The figure was adapted from [22], with permission.)

fairly wide tissue distribution in adults, including lymphoid, hematopoietic, epithelial and neural tissues [19,20]. But its expression is often limited to distinct cell types. In epithelia, for example, *bcl-2* is expressed within basal or proliferating cells that are not terminally differentiated [21]. Connections that are emerging between regulators of cell life and cell death therefore suggest that mechanisms of programmed cell death represent one more regulatory checkpoint in the normal life cycle of the cell.

Figure 5



Comparision between the three-dimensional structure of the transmembrane domain of diphtheria toxin [23] and Bcl-x_L. The transmembrane domain of diphtheria toxin contains two central helices consisting of apolar residues (orange); these helices are analogous to helices 5 and 6 of Bcl-x_L. In both proteins these central helices are surrounded by amphipathic helices (shown here in purple and white). Helix 1 and helices 3–6 of Bcl-x_L superimpose with the corresponding helices in diptheria toxin to 2 Å over 50 Cα positions.

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